

STUDIES ON THE DEVELOPMENT OF CHLOROPLAST PIGMENTS OF PLANTS

CARL G. DEUBER

- I. Influence of Mineral Elements upon Development of Chloroplast Pigments of Soy Beans. *Botanical Gazette*, Vol. LXXXII, No. 2, October, 1926. pp. 132-153.
- II. Can a Pyrrole Derivative be Substituted for Iron in the Growth of Plants? *American Journal of Botany*, XIII, 276-285. May, 1926.
- III. Potassium Ferrocyanide and Ferric Ferro-cyanide as Sources of Iron for Plants. *Soil Science*, Vol. XXI, No. 1. January, 1926. pp. 23-26.

NOTE—These papers are submitted to the Faculty of the University of Missouri as a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June, 1925.

**INFLUENCE OF MINERAL ELEMENTS UPON
DEVELOPMENT OF CHLOROPLAST
PIGMENTS OF SOY BEANS**

CARL G. DEUBER



Reprinted for private circulation from
THE BOTANICAL GAZETTE, Vol. LXXXII, No. 2, October 1926

PRINTED IN THE U.S.A.

INFLUENCE OF MINERAL ELEMENTS UPON DEVELOPMENT OF CHLOROPLAST PIG- MENTS OF SOY BEANS¹

CARL G. DEUBER

(WITH FIVE FIGURES)

Introduction

Cultural experiments with plants have demonstrated many instances in which deficiencies of various mineral elements have produced, in addition to reduced growth, a disturbance in the development of the chloroplast pigments. The commonest manifestation of such a condition is the yellow or white color of the foliage of plants lacking a proper supply of iron. Most of the other essential elements also influence the development of the chloroplast pigments.

VILLE (24), working with hemp plants, found that the greatest decrease in chlorophyll and carotin was brought about by a deficiency of nitrates. Lesser decreases resulted from deficiencies of potassium, phosphate, and calcium in the order named. LESAGE and SCHIMPER (22) noted that excesses of mineral substances reduced the chlorophyll content of plants. REED and HAAS (18) investigated the effects of several ions upon young citrus and walnut trees. They found that chlorosis of the leaves resulted from high applications of chlorine in the form of sodium chloride, from the application of sodium bicarbonate in solutions deficient in calcium, from high concentrations of potassium, and from very low concentrations of magnesium. GARNER and co-workers (7) described chloroses of tobacco plants caused by deficiencies of potassium, sulphur, and magnesium. MAMELI (14) found that within certain limits the development of the chlorophylls (*a* and *b*) was proportional to the magnesium supplied in the nutrient solution. The mottling of *Coleus* plants was associated with a shortage of nitrates by SCHERTZ (20). BOKORNY (2) observed that the first effect of cultivating green algae in solutions lacking calcium was a decrease in the chlorophyll content.

¹ Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Missouri, 1925.

Applications of sulphur-containing fertilizers to sweet corn (6), alfalfa (19), rutabagas, parsnips, and beets (3) resulted in a darker green color of the foliage. MCHARGUE (12) reported manganese in small amounts essential for proper chlorophyll development. MCLEAN and GILBERT (13) have corrected a chlorosis of spinach by spraying with manganous sulphate solutions.

While there is abundant evidence that various mineral elements influence the formation of the chloroplast pigments, very little has been accomplished in showing in what manner this influence is operative. A mineral element may function as a constituent of the chlorophylls as magnesium; it may act as a catalyst in the formation of specific portions of the pigment, as is suggested by ODDO and POLACCI (16) for iron; or it may not have a direct bearing on the pigments themselves but so influence the general metabolic processes, such as respiration, that the normal production of the chloroplast pigments is more or less impaired.

WILLSTÄTTER (25) has shown conclusively that magnesium is the only mineral constituent of chlorophyll (*a* and *b*). The reason for the connection of this element with chlorophyll development demonstrated by MAMELI, GARNER and co-workers, and by REED and HAAS is therefore clear. Nitrogen is also a constituent of chlorophyll. ODDO and POLACCI suggest that iron acts as a catalyst in the formation of the pyrrole groupings which are the basis of the chlorophyll molecules. These investigators arrived at this hypothesis from the successful substitution of a magnesium salt of pyrrole carbonic acid for iron in the growth of plants. Studies by the writer (4) with a similar compound substituted for iron with several plants gave negative results in all trials. We do not know in what way the other mineral elements are connected with the formation of the chloroplast pigments.

Not only is our knowledge limited as to the way in which the mineral elements are connected with the development of the chloroplast pigments, but we also know very little of the effect of an element upon the development of the individual pigments found in the chloroplast. Yet in view of present theories of photosynthesis (1) which associate all four of the chloroplast pigments with this process such information is important. By comparing the absorption bands

of chlorophylls (*a* and *b*) in living leaves, WLODEK (27) has been able to show that a lack of potassium causes an absolute and relative diminution of chlorophyll *b* and an increase in chlorophyll *a*. The data of MAMELI for maize show that increasing the magnesium supply above a certain point results in a decrease in the quantity of carotinoids, although the chlorophylls were further increased.

The work reported in this paper and elsewhere by the writer was planned to determine the effect of several essential mineral elements on the development of the chloroplast pigments, and to secure information on the method of their action. In the present investigation soy bean plants were grown in water cultures, and the influence of iron, potassium, and sulphur in the development of the chloroplast pigments studied by making quantitative determinations of the chlorophylls and the carotinoids.

Methods

The culture vessels employed were 500 cc. wide-mouth bottles wrapped in black paper and provided with paraffined brown paper tops. Three or four Wilson soy bean seedlings were grown in each culture. Iron wire rings with uprights were fastened to the culture vessels with rubber bands to support the tops of the plants. In the experiments with iron salts these supports were new, so that iron from iron rust was not a factor.

Knop's nutrient solution with the following composition was used: $\text{Ca}(\text{NO}_3)_2$, 0.8 gm.; KNO_3 , 0.2 gm.; KH_2PO_4 , 0.2 gm.; MgSO_4 , 0.2 gm.; distilled water, 1000 cc. Separate stock solutions of the constituent salts were prepared 100 times the concentration used in the cultures, dilution and mixing of the salts being made just before renewing the culture solutions every four or five days. Stock solutions of ferric citrate and ferrous sulphate were prepared fresh at each renewal of the solutions, to prevent hydrolysis of the iron salts. Merck's "reagent" and Baker's "analysed" chemicals were used, and the distilled water was obtained from a tripure still.

In the experiments with potassium, the basal Knop's solution was modified to contain 0, 28, 134, and 238 p.p.m. K. This element was omitted from the first solution by substituting NaH_2PO_4 for KNO_3 and KH_2PO_4 . The second solution contained 28 p.p.m. K in

the form of KH_2PO_4 , and the third with 134 p.p.m. K was the regular Knop's solution. The fourth solution contained 238 p.p.m. K in the forms of KNO_3 , KH_2PO_4 , and KCl.

In the experiment with sulphur, the basal Knop's solution was modified to contain 0, 13, 26, and 46 p.p.m. S. Sulphur was omitted from the first solution by replacing MgSO_4 with MgCl_2 . The second solution with 13 p.p.m. S received but half the MgSO_4 of the Knop's solution, the Mg content being maintained by adding MgCl_2 . Knop's solution contains 26 p.p.m. S, the concentration employed in the third solution. The fourth solution with 46 p.p.m. S received Na_2SO_4 in addition to the MgSO_4 of the Knop's solution. Iron was supplied to the solutions of the potassium and sulphur experiments in the form of ferric citrate at the rate of 0.228 p.p.m. iron.

The reactions of the nutrient solutions were determined at the time of preparing the solutions and after the plants had grown in them four or five days, the colorimetric method of GILLESPIE (8) being employed.

The experiments were conducted on central benches in a well lighted greenhouse having an average temperature of 20°C .

In the first experiments with iron and potassium, the chloroplast pigments were determined by extracting the leaves of the plants which had been air dried in the dark at approximately 18°C . In the later experiments the chloroplast pigments were extracted and estimated immediately upon harvesting the fresh plants. The methods of WILLSTÄTTER, modified as suggested by SCHERTZ² for the extraction and separation of the chloroplast pigments were followed. The procedure consisted in extracting the leaf material with acetone, transferring the acetone extract to ether, and saponifying the chlorophylls in order to separate them from the carotinoids. Carotin and xanthophyll were separated by means of their different solubilities in petroleum ether and methyl alcohol.

The acetone extracts of a series of leaf samples were compared relatively in a Dubosque colorimeter. The ether extracts containing

² The writer wishes to acknowledge his thanks to Dr. F. M. SCHERTZ, United States Bureau of Plant Industry, for suggestions regarding the extraction, separation, and quantitative estimation of the chloroplast pigments, and for a sample of purified chlorophyll (*a* and *b*) mixed.

all the chloroplast pigments were treated in the same way. The mixed chlorophylls (*a* and *b*) of a given series of samples were compared relatively and with alcoholic solutions of chlorophylls (*a* and *b*), the purified chlorophyll for these standards being furnished by SCHERTZ. Relative comparisons were made of the combined carotinoid samples and of the carotin and xanthophyll.

Influence of iron

Two experiments were performed on the influence of iron as ferrous sulphate and as ferric citrate, on the growth and on the chloroplast pigments of soy bean plants. In the first experiment Knop's solution containing iron as ferrous sulphate at rates of 0.367, 0.734, 1.468, 2.936, 4.411, 5.872, and 8.822 p.p.m., and iron as ferric citrate at rates of 0.228, 0.456, 0.912, 1.824, 2.736, 3.648, and 5.472 p.p.m. Two cultures of three plants each were used for each concentration of iron. The solutions were renewed every four days. The total quantity of iron supplied per plant in milligrams was 1.5 times the concentration in parts per million. The initial hydrogen-ion concentration varied from P_H 5.65 to 5.8, and the maximum final from P_H 6.15 to 6.7, the greater changes occurring with the larger plants.

After twelve days' growth, chlorosis of the new leaves in both series of iron salts was evident with the first three concentrations of iron and the solution lacking iron. The chlorotic condition was soon followed by a black spotting of the youngest leaves. This condition first appeared in the form of minute yellow areas in the leaf blades; these increased to irregular shaped areas less than 1 mm. in size, and underwent changes in color to light red, red, and finally to reddish brown or black. Young leaves were often peppered over their entire surface with these spots and dropped off prematurely. This condition was most evident in the ferrous sulphate series. At the conclusion of the experiment, after 35 days' growth, chlorosis was evident in the first five solutions of the ferrous sulphate series and in the first four of the ferric citrate series.

The growth of the plants was markedly affected by the concentration or amount of iron supplied.³ Increasing the concentration of

³ It is impossible to differentiate in these experiments between concentration and total amount of an element supplied. In a given series the nutrient solutions were

iron as ferric citrate produced improved growth up to 3.648 p.p.m. (fig. 1). Increasing iron as ferrous sulphate increased growth up to 8.822 p.p.m., the maximum used.

Ferric citrate proved to be a much more efficient source of iron than ferrous sulphate at all concentrations employed. A comparison of the growth curves of fig. 1 makes it possible to estimate the

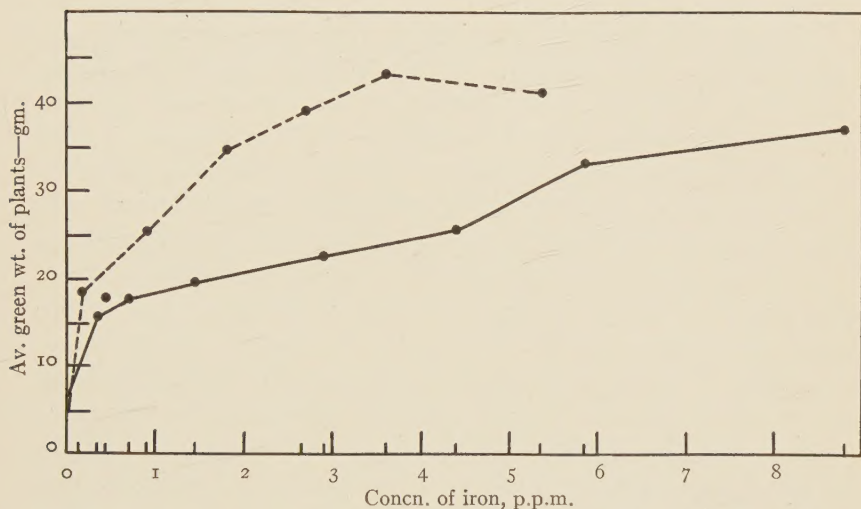


FIG. 1.—Green weights of soy bean plants per culture supplied seven concentrations of iron as ferrous sulphate and ferric citrate (p.p.m.=parts per million). Dotted line, ferric citrate; continuous line, ferrous sulphate.

quantity of iron as ferric citrate which would be required to produce the same amount of growth obtained with the various concentrations of ferrous sulphate iron. Thus to produce 37.155 gm. fresh weight of plants secured with 8.822 p.p.m. iron as ferrous sulphate would require 2.3 p.p.m. ferric citrate iron, or 3.8 times as much iron as ferrous sulphate as in the form of ferric citrate. For a concentration of 5.872 p.p.m. iron as ferrous sulphate this factor is 3.5; for a concentration of 4.411 p.p.m., 4.4; for a concentration of 2.936 p.p.m., 4.5; and for a concentration of 1.468 p.p.m. it is 4.9. In other words,

changed the same number of times. The total quantity of an element supplied varied with the concentration. When the term concentration is used in this paper it is understood that total quantity or amount of the element supplied may be the governing factor and not the concentration.

iron as ferric citrate in the range of concentrations where marked depression of growth did not occur was about four times as efficient in this experiment as iron in the form of ferrous sulphate. Since the highest concentration of ferrous sulphate employed produced no decrease in growth its optimum concentration cannot be stated, but for ferric citrate a concentration of 3.648 p.p.m. iron was the optimum.

Attempts to determine the plastid pigments were made by extracting with acetone two gm. samples of leaves dried as previously described. When the green pigments of the acetone extracts were transferred to ether, some of the greenest leaf samples gave a very green wash water, which would not give up its coloring matter to ether, petroleum ether, or carbon bisulphide. More pigment was transferable to ether from the acetone extracts of some of the chlorotic leaf samples than from that of some of the greenest and most vigorous plants. Evidently, during the drying of the leaves some of the chloroplast pigments were decomposed to a water soluble form. Comparisons of the total pigment material extracted from dried leaves by 80 per cent acetone appeared unreliable. In the ferric citrate series the highest pigment content was found in the plants grown in a solution containing 0.456 p.p.m. iron. These plants had yellow to light green leaves. From these results it was concluded that acetone extracts of dried soy bean leaves are unsafe criteria for the estimation of the chloroplast pigments, and that satisfactory quantitative determinations of the chloroplast pigments may be seriously interfered with by changes in the pigments occurring during the drying of the leaves. Although WILLSTÄTTER (11) used the dried powder of leaves for all ordinary extractions, he found that drying caused a loss of chlorophyll, and when the leaves were not properly dried the pigments were altered. SCHERTZ strongly advises using fresh leaves for all quantitative pigment extractions.

In the second experiment soy bean plants were grown thirty days (January 18–February 17, 1925) in Knop's solution supplied iron as ferrous sulphate at rates from 0.367 to 7.340 p.p.m. iron, as indicated in table I, and in the form of ferric citrate at rates from 0.228 to 4.560 p.p.m. as given in table II. Four cultures of four plants each were employed for each concentration of iron. The solutions were

renewed every five days. The total quantity of iron supplied in mg. per plant was 0.83 times the concentration in p.p.m. The initial hydrogen-ion concentration of the solutions varied from P_H 5.5 to 5.7.

The foliage of the plants in solutions 1 and 2 of both series became chlorotic. The black spot condition of the leaves was most pronounced in solutions 1, 2, and 3 of the ferrous sulphate series. Immediately upon determining the green weights of the plants, the pigments of 10 gm. samples of the fresh leaves were extracted with acetone.

TABLE I

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, FERROUS SULPHATE SERIES

	SOLUTION AND LEAF SAMPLE NUMBER				
	1	2	3	4	5
Concentration of iron, p.p.m.	0.000	0.367	1.835	3.670	7.340
Green weight in gm.	7.705	10.423	13.158	13.651	14.925
Ether extract of chlorophyll and carotinoids	0.78	1.00	1.00	1.02	0.95
Chlorophyll (<i>a</i> and <i>b</i>)*	0.0045	0.0104	0.0134	0.0137	0.0149
Total carotinoids	0.55	1.00	0.64	1.00	0.74
Carotin	0.55	1.00	0.97	1.42	1.48
Xanthophyll	0.52	1.00	0.87	1.18	0.97

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

The chloroplast pigments were transferred without loss from acetone to ether, and the relative values of the combined chlorophylls and carotinoids determined by comparison with those of sample 2 of each series. The chlorophyllin salts were secured and compared with an alcoholic solution of 0.257 gm. of purified chlorophyll (*a* and *b*) in 250 cc. The combined carotinoids, carotins, and xanthophylls were then compared relatively with those of sample 2. The values for these determinations are summarized in tables I and II.

Ferric citrate was again the most efficient source of iron, but in this experiment the order of efficiency was much higher than in the previous one. For solution 5 the iron of ferric citrate was 13.3 times as efficient as that of ferrous sulphate, for solution 4, 14.7, and for

solution 3, 12.2. The optimum concentration of iron as ferric citrate was 2.280 p.p.m., but with ferrous sulphate the highest concentration employed (7.340 p.p.m.) gave the most growth.

With both ferrous sulphate and ferric citrate an increase in the iron supply to the plants resulted in increases of the chlorophylls of a unit of fresh leaf material. An exact proportional relation did not exist between the iron supplied and the amount of chlorophyll found per unit of leaf material. A very good correlation appears to exist between the chlorophyll content per unit of fresh leaves and growth,

TABLE II

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, FERRIC CITRATE SERIES

	SOLUTION AND LEAF SAMPLE NUMBER				
	1	2	3	4	5
Concentration of iron, p.p.m. . .	0.000	0.228	1.110	2.280	4.560
Green weight in gm.	7.984	13.968	16.988	18.732	17.928
Ether extract of chlorophyll and carotinoids	0.38	1.00	1.26	1.53	1.60
Chlorophyll (<i>a</i> and <i>b</i>)*.	0.0058	0.0087	0.0138	0.0160	0.0160
Total carotinoids.	0.46	1.00	1.00	1.07	1.47
Carotin	0.22	1.00	1.11	1.21	1.53
Xanthophyll.	0.34	1.00	0.96	1.21	1.49

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

as can readily be seen from the curves of fig. 2. The values of the chlorophylls (*a* and *b*) in terms of purified chlorophyll (*a* and *b*) almost equal the growth of the plants when multiplied by 1000, except with the samples from plants grown without iron.

The values of the total chloroplast pigments in the ether extracts and the carotinoids present some relations that differ from the chlorophyll values. In the ferrous sulphate series the total chloroplast pigments from leaves of plants grown in solution 1 show a marked diminution, but those of solutions 2, 3, 4, and 5 give practically the same values. The leaves of the plants in solution 2 were light green, while those in solutions 3, 4, and 5 were normal green. Why this difference was not evident in the ether extracts cannot be explained. In the ferric citrate series the pigments in the ether ex-

tracts increased in their relative values from solution 1 to 5, which would appear logical judging from the values of the chlorophylls. The combined carotinoids of samples 2 and 4 of the ferrous sulphate series, and samples 3, 4, and 5 of the ferric citrate series exhibited a greenish cast which could not be removed by washing with distilled water, 1 per cent sodium carbonate solution, or by filtering through

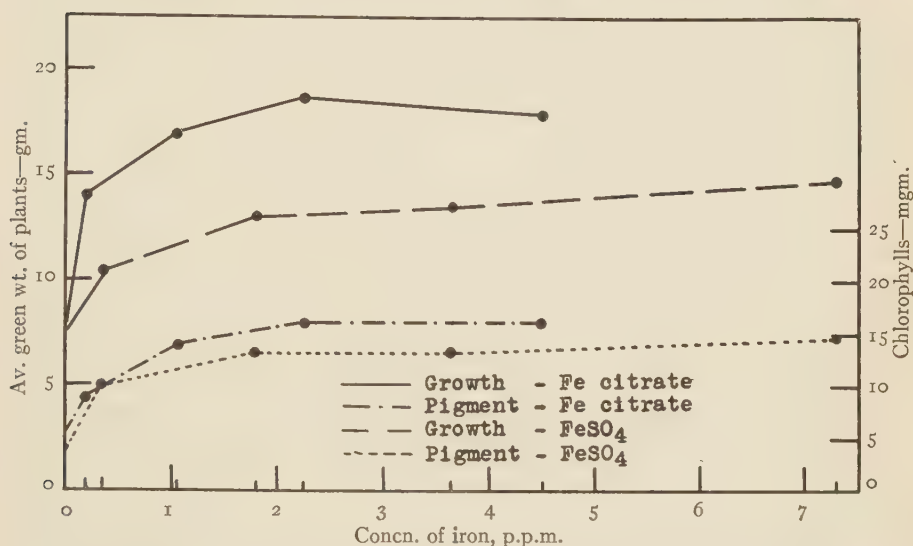


FIG. 2.—Green weights of soy bean plants per culture supplied four concentrations of iron as ferrous sulphate and ferric citrate, and amounts of chlorophyll (*a* and *b*) produced per 10 gm. fresh leaves; chlorophyll (*a* and *b*) expressed in terms of purified chlorophyll (*a* and *b*).

anhydrous sodium sulphate. This made it difficult to make accurate relative comparisons.⁴ The separated carotins and xanthophylls of both series exhibited increases with increased iron supply except in the case of sample 5, ferrous sulphate series, where a reduction was found.

Influence of potassium

Two experiments were performed on the influence of potassium on the growth, and on development of the chloroplast pigments of soy bean plants. In the first experiment the plants were grown

⁴ SCHERTZ suggested that the greenish cast of the carotinoid solutions might result from the incomplete saponification of the ether extracts.

thirty-six days (October 12-November 17, 1924) in Knop's solution lacking potassium and containing 28, 134, and 238 p.p.m. potassium. Solution 3 with a concentration of 134 p.p.m. potassium, the normal Knop's solution, was considered the control solution. Each solution was replicated with four cultures of four plants each. The solutions were changed every four days.

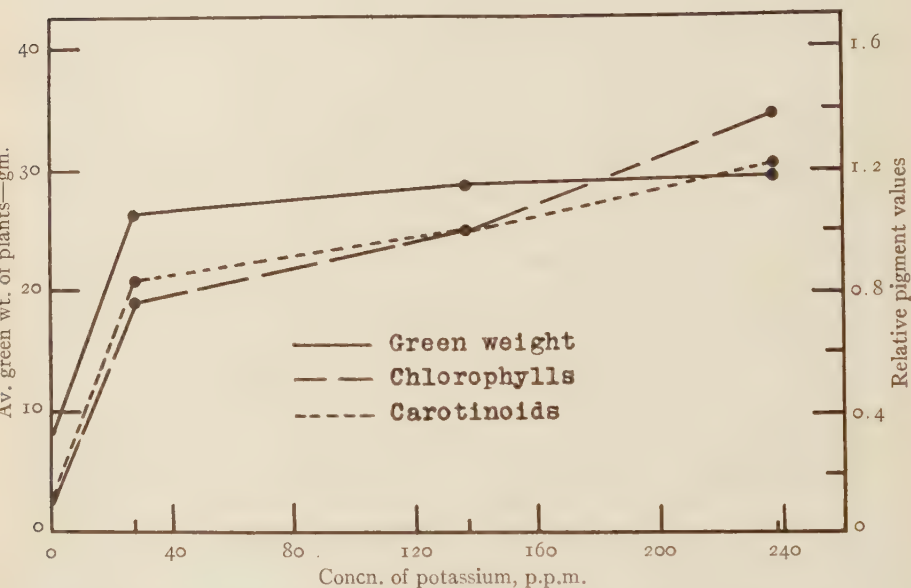


FIG. 3.—Green weights of soy bean plants per culture supplied three concentrations of potassium, and relative values of chlorophyll (*a* and *b*) and carotinoids of 2 gm. samples of dried leaves.

The growth of the plants in the solution lacking potassium was very much stunted, and the leaves developed large translucent areas on the blades and dark red areas along the stems. The plants in the solutions containing potassium made rapid growth. Only small increases in green weight were secured with additions of potassium above 28 p.p.m. (fig. 3). No differences in color of the leaves were observable between the plants in the low and high potassium-containing solutions.

The tops of the plants were air dried in the dark and the pigments extracted from 2 gm. samples of the leaves. In sample 1 some

difficulty was experienced in transferring all the green pigment to ether from the acetone extract. The relative values of the four chloroplast pigments, the chlorophylls, and the carotinoids in ether were determined by comparison with those of sample 3, which were given a value of 1.0. The chlorophyllins were also compared with a solution of 0.0025 gm. of purified chlorophyll (*a* and *b*) in

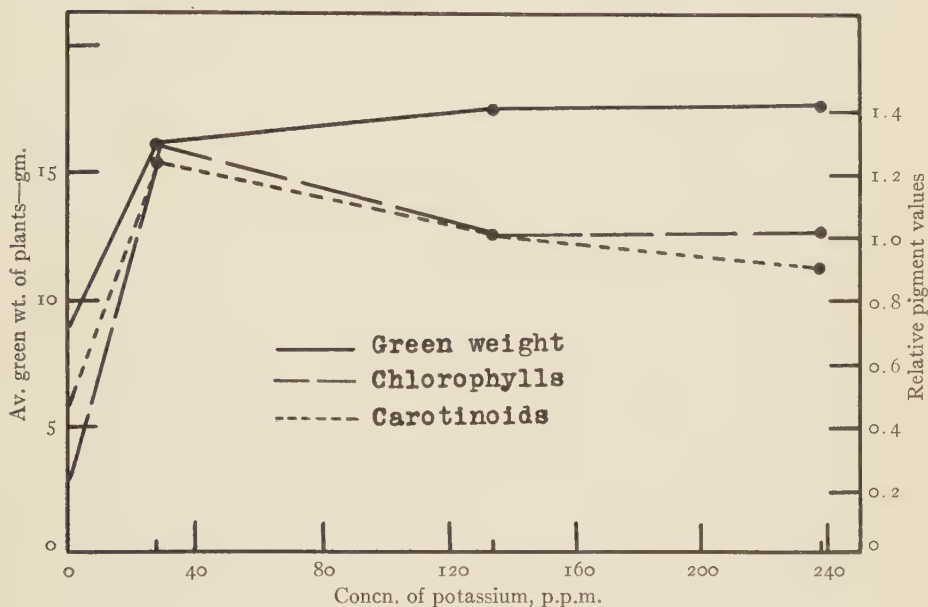


FIG. 4.—Green weights of soy bean plants per culture supplied three concentrations of potassium, and relative values of chlorophyll (*a* and *b*) and carotinoids of 10 gm. samples of fresh leaves.

100 cc. of alcohol. The results of these determinations are shown in fig. 3.

The chloroplast pigments of the leaves were greatly depressed when potassium was withheld from the plants, but the addition of 28 p.p.m. potassium to the nutrient solution resulted in a very large increase in these pigments. Rates of 134 and 238 p.p.m. potassium produced further increases in all the pigments except the xanthophyll of sample 4, which was lower than that of sample 3.

The extraction of the chloroplast pigments from the dried leaf samples in this case was apparently satisfactory. Observable loss of

the chloroplast pigments occurred only in sample 1. This was in distinct contrast to the results secured with the dried leaves of the series with iron. The difference can probably be accounted for by differences in the rapidity of drying of the two lots of leaves, although both were dried in the dark at room temperatures. Although no loss of pigment, with the exception noted, was observed in the wash water from the acetone extracts, the chlorophyll found in the dried leaves was much less than found later in fresh leaves. Expressing the chlorophylls in terms of purified chlorophyll (*a* and *b*), the maximum found in this series in 2 gm. of dried leaves was 5.3 mg., and in the second series with potassium in 10 gm. of fresh leaves the maximum was 18.0 mg. This would suggest a considerable loss of pigments during the drying process, assuming that the leaves contained approximately 80 per cent moisture.

The second experiment with potassium was a replicate of the one already described, and was performed to secure fresh leaves for the extraction of the pigments. The cultures contained four plants each. The solutions were changed every five days during a growing period of thirty days (January 14–February 13, 1925).

At the end of the experiment the plants in the solution lacking potassium were stunted, the leaves crinkled with large white areas in the blades, while some of the older leaves were turning yellow. Many of the stems exhibited dark red to brown areas. The new leaves of the plants in the solution containing 28 p.p.m. potassium were yellowish green and crinkled. The solutions with the highest concentrations of potassium, 134 and 238 p.p.m., produced plants that were normal in every respect.

The pigments of 10 gm. samples of the fresh leaves were extracted immediately upon harvesting the plants. The ether extracts containing the chlorophyll (*a* and *b*) and carotinoids, and the combined carotin and xanthophyll were compared relative to those of sample 3. The chlorophylls were compared with a solution of 0.0257 gm. purified chlorophyll in 250 cc. of alcohol. A summary of these determinations is given in table III.

The chloroplast pigments were markedly reduced when potassium was omitted from the nutrient solution, but showed a very great increase with a concentration of 28 p.p.m. of this element. The

values obtained for sample 2 appear to be too high when compared with those of samples 3 and 4, because at the time of harvesting the plants many of the leaves in solution 2 were slightly chlorotic, while those in solutions 3 and 4 were normally green. The series in which dried leaves were used for extraction also shows no such high chlorophyll content for the plants in solution 2. Duplicate samples of the fresh leaves in the second series gave similar high results for sample 2. The chlorophylls of sample 4 when compared with a solution of purified chlorophyll (*a* and *b*) were greater than those of sample 3, but relative comparisons of these pigments gave approximately the

TABLE III

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, SECOND POTASSIUM SERIES

	SOLUTION AND LEAF SAMPLE NUMBER			
	1	2	3	4
Concentration of K, p.p.m.....	0.000	28 ^a	134	238
Green weight in gm.....	8.995	16.178	17.722	17.756
Ether extract of chlorophyll and carotinoids.....		1.20	1.00	0.95
Chlorophyll (<i>a</i> and <i>b</i>).....	0.22	1.32	1.00	1.07
Chlorophyll (<i>a</i> and <i>b</i>)*.....	0.0030	0.0180	0.0136	0.0146
Total carotinoids.....	0.48	1.25	1.00	0.90

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

same values. The carotinoids of sample 4 showed a slight decrease as compared with those of sample 3.

Influence of sulphur

Soy bean plants were grown in solutions lacking sulphur and containing 13, 26, and 46 p.p.m. of this element. Four plants were placed in each culture and the cultures replicated four times for each solution. The solutions were renewed every five days during a growing period of forty-three days (January 19–March 3, 1925).

A slight chlorosis of the new leaves of the plants in the solution containing the highest concentration of sulphur (46 p.p.m.) occurred in the fourth week, but by the end of the growing period these leaves were normal green. Some of the leaves were crinkled and developed

white to rust colored areas in the blades, together with a drying of the leaf margins. A similar crinkling of new leaves and discolored areas in the blades were also found in the plants deprived of sulphur. A swelling of lateral roots was noted in the plants lacking a supply of sulphur and those receiving the lowest concentration of this element.

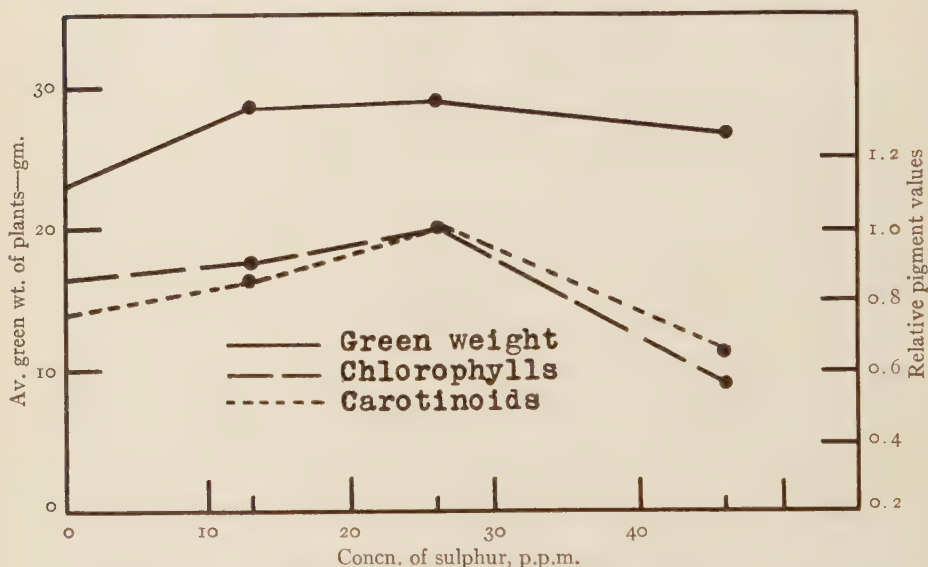


FIG. 5.—Green weights of soy bean plants per culture supplied three concentrations of sulphur, and relative values of chlorophyll (*a* and *b*) and carotinoids of 10 gm. samples of fresh leaves.

The growth of the plants was only slightly influenced by the concentration or amount of sulphur supplied in the nutrient solutions, as indicated by the green weight data of table IV. DUGGAR (5) states that sulphur is usually required in such limited quantity that the seeds may furnish all that is needed for normal growth of plants through a considerable period. HART and PETERSON'S (9) data on the sulphur content of a number of seeds show that soy bean seeds are high in this element. Soy beans contain 0.341 per cent sulphur, wheat 0.170, corn 0.164, and oats 0.189 per cent.

The chloroplast pigments of 10 gm. samples of fresh leaves were extracted and determined relatively. These data are given in table

IV. The maximum development of the chloroplast pigments per unit of fresh leaves was found in the plants grown in the regular Knop's solution (solution 3) with sulphur at a concentration of 26 p.p.m. A decrease or an increase in the sulphur supplied as compared with that of Knop's solution decreased the quantity of chloroplast pigments, the excess sulphur supply causing the greater depression.

TABLE IV

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, SULPHUR SERIES

	SOLUTION AND LEAF SAMPLE NUMBER			
	1	2	3	4
Concentration of S, p.p.m.....	0.000	13	26	46
Green weight in gm.....	23.340	28.517	28.959	26.949
Ether extract of chlorophyll and carot- tinoids.....	0.71	0.83	1.00	0.58
Chlorophyll (<i>a</i> and <i>b</i>).....	0.86	0.90	1.00	0.57
Chlorophyll (<i>a</i> and <i>b</i>)*.....	0.0092	0.0094	0.0107	0.0058
Total carotinoids.....	0.76	0.86	1.00	0.65
Carotin.....	1.03	0.81	1.00	0.46
Xanthophyll.....	0.98	0.80	1.00	0.46

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

Discussion

Of the three mineral elements studied, the lack of iron was found to limit the growth of soy bean plants most, followed by potassium. Sulphur had a relatively slight influence on the growth of these plants. The source of the iron presented some interesting features in connection with growth. Ferric citrate in the first experiment proved to be about four times as efficient a source of iron as ferrous sulphate, and in the second experiment about thirteen times as efficient. The difference in the value of this factor for the two experiments is probably due to differences in environment, and to the fact that one series was grown for five weeks and the other for four weeks. The relative constancy of this factor of efficiency in each of these experiments indicates a constant difference in the availability of iron in the citrate and sulphate. The cause of the difference in efficiency of the iron in these two salts may be due to a lower solubility of the

ferrous sulphate, the ferric or ferrous state of the iron or the anion with which the iron is combined. Of these possible causes, that of solubility appears to be the most probable. TOTTINGHAM and RANKIN (23) found that the solubility of iron as ferric citrate was about four times that of iron as ferrous sulphate in the Livingston-Tottingham solution R_8C_1 at a P_H of 6.0. A similar constant relation can also be found in the data of JONES and SHIVE (10) with ferric phosphate and ferrous sulphate in the growth of wheat. Ferrous sulphate was approximately 1.6 times more efficient than the less soluble ferric phosphate.

The optimum concentrations of ferric citrate were 2.280 and 3.648 p.p.m. iron, but the highest concentrations of ferrous sulphate employed (7.340 and 8.822 p.p.m. iron) produced no decrease in growth, so the optimum concentration was not attained. MARSH and SHIVE (15) obtained the highest yield of soy bean plants with ferrous sulphate at the rate of 19.4 mg. iron per liter for three plants, or 6.4 mg. per plant over a growing period of five weeks when the iron supply was adjusted at frequent intervals. In the present experiments a supply of 13.233 and 6.116 mg. iron as ferrous sulphate per plant, over growing periods of five and four weeks respectively, gave very satisfactory growth.

The value of the determination of the chloroplast pigments depends upon the reliability of the methods used for such determinations. The operation is tedious and complicated, and there are numerous opportunities for error. It was found that the pigments of fresh leaves could be extracted much more easily and completely than those from dried ones. The acetone extracts from dried leaves often contained water-soluble green pigment which could not be transferred to ether. Determinations of the separated chlorophyllins from such extracts gave values that were obviously too low, which indicated a decomposition of the pigments during drying. Relative comparisons of 80 per cent acetone extracts of the total pigment content of leaf samples were not satisfactory, because of the presence of water soluble pigments. A comparison of the total chloroplast pigments by transferring from acetone to ether and washing out all water soluble material has the double disadvantage of permitting relative determinations only and of including both yellow and green

pigments. The comparison of the chlorophylls with known solutions of chlorophyll (*a* and *b*) gave the most satisfactory results, because the colors were comparable, and because, while not expressing exactly the quantity of chlorophyll present, it did allow a comparison of the amount of these pigments among different experiments. The carotinoids combined and separated were particularly difficult to determine accurately. A greenish tint impossible to remove in some of the carotinoid extracts made relative comparisons difficult. The cause of this variation in color could not be determined. The addition of concentrated sulphuric acid to a carotinoid extract in petroleum ether produces a clear green color, the original yellow color returning upon dilution with water. It is possible that a partial change of the carotinoids to this greenish form may occur during some stage in the manipulation. SCHERTZ is of the opinion that the greenish tint is the result of incomplete saponification of chlorophyll (*a* and *b*) altered probably by drying. Nevertheless, certain facts of interest appear evident from the determinations of the chloroplast pigments. The omission of iron or potassium from the solutions reduced the amount of the chloroplast pigments decidedly more than the omission of sulphur. This, however, does not mean that if sulphur were seriously deficient the pigment production would not be affected. The relatively high sulphur content of soy bean seeds probably supplies the seedlings with enough for a considerable period. The general tendency was for the pigments to increase with increasing supplies of iron and sulphur, but with the highest concentration of sulphur (46 p.p.m.) a very decided decrease in pigment content resulted. Judging from the results secured from the fresh leaves, the higher concentrations of potassium also caused a decrease in the amount of the chloroplast pigments.

One rather unexpected feature of the analyses is the fact that the carotinoids and chlorophylls vary for the most part together. A reduction in the chlorophylls is associated with a similar reduction in the carotinoids. This suggests that the formative processes involved in the production of these two types of pigments are influenced similarly by the essential elements included in this study. It also suggests that the yellowish colors frequently observable in chlorotic leaves are not due to carotin or xanthophyll but to xan-

thones, flavones, or related yellowish pigments. There is no evidence from the data to indicate that the formation of the chlorophylls is more dependent upon iron, potassium, or sulphur than the formation of the carotinoids. On the contrary, the determinations emphasize the close relationship which appears to exist between these two groups of chemically different pigments.

Summary

1. Soy bean plants were grown in nutrient solutions in which the concentrations of the iron, potassium, and sulphur contents were varied.

2. Ferric citrate was found to be four to thirteen times more efficient an iron source than ferrous sulphate. This difference in efficiency was constant in a given experiment.

3. Small differences in the iron content of the nutrient solutions produced considerable differences in the growth of the plants.

4. Iron deficiency caused small black spots to develop in the chlorotic new leaves.

5. Lack of potassium stunted growth markedly, but concentrations of 28-238 p.p.m. of this element produced about the same amount of growth.

6. The sulphur content of the nutrient solution influenced growth only slightly.

7. Acetone extracts of the total pigments of leaves are not safe criteria for judging the relative chloroplast pigment development.

8. Ether extracts of the chloroplast pigments freed from water soluble pigments are more satisfactory for judging the relative chloroplast pigment content than acetone extracts.

9. The separated carotinoid pigments may show greenish tinges which interfere with their quantitative estimation.

10. Estimation colorimetrically of chlorophyll (*a* and *b*) against a standard of purified chlorophyll (*a* and *b*) in alcohol was found to be a very satisfactory method.

11. The lack of iron or potassium in the nutrient solution resulted in a more marked depression of the chloroplast pigments than a lack of sulphur.

12. In general, the chloroplast pigments increased with the in-

creasing concentrations of iron and sulphur. The highest concentration of sulphur used (46 p.p.m.) caused a marked decrease in the pigment content of the leaves.

13. Concentrations of potassium above 28 p.p.m. reduced the chloroplast pigments when the extractions were made from fresh leaves.

14. In all the experiments the chlorophylls (*a* and *b*) and carotinoids were influenced by the composition of the nutrient solution to about the same extent.

15. An exact proportional relation could not be established between the concentration or amount of any of the elements studied and the amount of pigment formed.

16. A correlation between the green weight of the plants and the chlorophyll (*a* and *b*) existed in one experiment with iron, but did not apply in the other experiments.

The writer desires to express his sincere thanks to Dr. W. J. ROBBINS, of the University of Missouri, for advice and constructive criticism throughout the prosecution of this investigation and in the preparation of the manuscript, and to Dr. F. M. SCHERTZ, United States Bureau of Plant Industry, for samples of purified chlorophyll (*a* and *b*), for many suggestions in regard to methods of procedure, and for advice in the presentation of the pigment data.

YALE UNIVERSITY
NEW HAVEN, CONN.

[Accepted for publication December 2, 1925]

LITERATURE CITED

1. BALY, E. C. C., Photosynthesis. *Nature* **109**:344-346. 1922.
2. BOKORNY, T., Über den Einfluss des Calciums und Magnesiums auf die Ausbildung der Zellorgane. *Bot. Centralbl.* **62**:1-4. 1895.
3. DEMOLON, M. A., Sur l'action fertilisante du soufre. *Compt. Rend. Acad. Sci. Paris* **154**:524-526. 1912.
4. DEUBER, C. G., Can a pyrrole derivative be substituted for iron in the growth of plants? *Amer. Jour. Bot.* **13**:276-285. 1926.
5. DUGGAR, B. M., Plant physiology with special reference to plant production. pp. 516. New York: Macmillan Co. 1917.
6. DULEY, F. L., Relation of sulphur to soil productivity. *Jour. Amer. Soc. Agron.* **8**:154-160. 1916.

7. GARNER, W. W., McMURTREY, J. E., BACON, C. W., and MOSS, E. G., Sand drown, a chlorosis of tobacco due to magnesium deficiency and the relation of sulphates and chlorides of potassium to the disease. *Jour. Agric. Res.* **23**:27-40. 1923.
8. GILLESPIE, L. J., Colorimetric determination of the titration curves without buffer mixtures. *Jour. Amer. Chem. Soc.* **42**:742-746. 1920.
9. HART, E. B., and PETERSON, W. H., Sulphur requirements of farm crops in relation to the soil and air supply. *Wis. Agric. Exp. Sta. Res. Bull.* **14**. 1-21. 1911.
10. JONES, L. H., and SHIVE, J. W., The influence of iron in the forms of ferric phosphate and ferrous sulphate upon the growth of wheat in nutrient solutions. *Soil Sci.* **11**:93-99. 1921.
11. JØRGENSEN, I., and STILES, W., Carbon assimilation. *New Phytol.* **14**:281-294. 1915.
12. MCHARGUE, J. S., Effect of different concentrations of manganese sulphate on the growth of plants in acid and neutral soils and the necessity of manganese as a plant nutrient. *Jour. Agric. Res.* **24**:781-794. 1923.
13. MCLEAN, F. T., and GILBERT, B. E., Manganese as a cure for a chlorosis of spinach. *Science* **61**:636-637. 1925.
14. MAMELI, E., Sulla influenza del Magnesio sopra la formazione della Chlorofilla. *Univ. di Pavia, Atti Ist. Bot.* **2/15**:151-205. 1918.
15. MARSH, R. P., and SHIVE, J. W., Adjustment of iron supply to requirements of soy beans in solution culture. *BOT. GAZ.* **79**:1-27. 1925.
16. ODDO, B., and POLACCI, G., Influenza del nucleo pirrolico nella formazione della chlorofilla. *Gaz. Chim. Ital.* **50**:54-70. 1920.
17. PALMER, L. S., Carotinoids and related pigments. pp. 316. New York: 1922.
18. REED, H. S., and HAAS, A. R. C., Nutrient and toxic effects of certain ions on citrus and walnut trees with especial reference to the concentration and P_H of the medium. *Univ. Calif. Publ. Tech. Paper no. 17*. 1-75. 1924.
19. REIMER, F. C., and TARTAR, H. V., Sulphur as a fertilizer for alfalfa in southern Oregon. *Ore. Agric. Exp. Sta. Bull.* **163**. 1-40. 1919.
20. SCHERTZ, F. M., A chemical and physiological study of mottling of leaves. *BOT. GAZ.* **71**:81-130. 1921.
21. ———, The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. *Jour. Agric. Res.* **26**:383-400. 1923.
22. SCHIMPER, A. F. W., Die Indo-Malayische Strandflora. Jena. 1891 (cited by PALLADIN, V. I., *Plant physiology* p. 17. 1923).
23. TOTTINGHAM, W. E., and RANKIN, E. J., The availability of iron in nutrient solutions. *Amer. Jour. Bot.* **10**:203-210. 1923.
24. VILLE, G., Recherches sur les relations qui existent entre la couleur des plants et la richesse des terres en agents de fertilité. *Compt. Rend. Acad. Sci. Paris* **109**:397-400. 1889.

25. WILLSTÄTTER, R., Zur Kenntniss der Zusammensetzung des Chlorophylls. Liebig's Ann. Chem. **350**:48-82. 1906.
26. WILLSTÄTTER, R., and STOLL, A., Untersuchungen über chlorophyll. pp. 424. Berlin. 1913.
27. WLODEK, J., Recherches sur l'influence des engrais chimiques sur le coefficient chlorophyllien. Bull. Acad. Polonaise Sci. et Lettres Classe Sci. Math. et Nat., Ser. B **1920**:19-52. 1922 (cited in Bot. Absts. **12**:1095-1096. 1923).

CAN A PYRROLE DERIVATIVE BE SUBSTITUTED FOR IRON IN THE GROWTH OF PLANTS?

C. G. DEUBER

(Received for publication July 21, 1925)

In a preliminary note Pollacci and Oddo (4) discuss the presence of iron in the pigment of vertebrate blood and that of magnesium in the chlorophyll of green plants, and the fact that these metals are bound to organic complexes which yield similar pyrrole compounds on reduction. An experiment was described in which corn seedlings were grown in a nutrient solution lacking iron and in one containing a magnesium salt of pyrrole carbonic acid in place of iron. After 20 days' growth the corn plants in the solution containing the pyrrole salt were normally green and three times the size of the chlorotic plants grown without iron. In a more extensive paper (3) these investigators report normal growth and chlorophyll-development of 6 species of plants in solutions containing this pyrrole salt but lacking iron. From these experiments and from the analytical work of Willstätter (5, 6) upon the chemistry of chlorophyll the theory was advanced that iron functions as a catalyst in the formation of pyrrole groupings which go to make up the nucleus of the complex chlorophyll molecule. The Italian workers contend that, when the pyrrole group is supplied to plants in a form which can be used, iron becomes unnecessary for the formation of chlorophyll.

The writer became interested in this subject through efforts to induce chlorophyll-development in some genetically yellow corn strains. It was soon apparent that considerable work would have to be done to secure positive results with normally green plants with a pyrrole salt substituted for iron. The main object of the present investigation therefore became an attempt to confirm the work of Pollacci and Oddo.

The literature dealing with the physiological action of pyrrole compounds in relation to plants is very limited. Ciamician and Galizzi (1) report that pyrrole carboxylic acid and dimethyl-pyrrole dicarboxylic acid are not toxic to plants. R. Emerson of Harvard University, in a communication to the writer, states that 2-4-dimethyl-3-5-dicarbothoxy-pyrrole and 2-phenoxy-pyrrole have been used in nutrient solutions for the growth of several species of plants with varying success.

The magnesium salt of pyrrole carbonic acid used in these experiments, except for a small sample received from Prof. Oddo, was prepared by J. S. Chu¹ and the writer in the Organic Chemistry Laboratory of the

¹ The writer wishes to acknowledge the assistance of Mr. J. S. Chu in the synthesis of the pyrrole salt and for making the analysis given above.

University of Missouri. The methods of Oddo and Moschini (2) for the synthesis of α -pyrrole carbonic acid were followed, and its magnesium salt was made according to the method of Oddo and Pollacci (3). The compound was purified by boiling with absolute alcohol. Melting-point determinations resulted in the compound's changing color and decomposing with the liberation of pyrrolic vapors between 255° and 260° C. Oddo and Pollacci state that the decomposition of the salt occurs at 260° C. in a closed tube.

An analysis of the compound was made with the following results:

	Found	Calculated
Mg.	9.81%	9.95%
N as NH_3	10.26%	12.68%
Loss on ignition.	83.98%	83.51%
Moisture.	23.10%	14.77%

The magnesium was determined by ignition of the compound to magnesium oxid, conversion to magnesium sulfate with sulfuric acid, and the titration of the excess sulfuric acid with sodium hydroxid. The nitrogen was determined as ammonia by the Kjeldahl method, and the moisture content by heating in an oven at 113° C. The results of these determinations indicate agreement in the composition of the compound with the theoretical in all respects except moisture. The analysis shows the presence of 3 molecules of water, while Oddo and Pollacci report 2 molecules of water attached to the compound.

The cultural studies with this pyrrole salt were carried out as far as possible in the same manner as described by the Italian workers. The nutrient solutions employed by them were used, consisting of solution *A*, which lacked iron and the pyrrole salt, and solution *B*, lacking phosphorus and iron but containing the pyrrole salt. A solution *C* was also used which contained in addition to the salts of solution *A* an iron salt, in order to compare the efficiency of the pyrrole salt with iron, a comparison which the Italian workers have failed to make. The compositions of the solutions were as follows:

Solution A		Solution B	
$\text{Ca}(\text{NO}_3)_2$	1.00 g.	$\text{Ca}(\text{NO}_3)_2$	1.00 g.
$(\text{NH}_4)_2\text{SO}_4$	0.25 g.	$(\text{NH}_4)_2\text{SO}_4$	0.25 g.
KNO_3	1.00 g.	KNO_3	1.00 g.
KH_2PO_4	0.25 g.	Pyrrole salt.	
Dist. water.	1,000 cc.	Dist. water.	1,000 cc.

GROWTH OF REID'S YELLOW DENT CORN WITH THE MAGNESIUM SALT OF PYRROLE CARBONIC ACID SUBSTITUTED FOR IRON

Reid's Yellow Dent corn seedlings were grown in solution *A* minus iron, solution *B* with the pyrrole salt at the rate of 0.125 g. per liter, and in solution *C* containing 0.03 g. of ferric chlorid per liter. Since it was not understood why Oddo and Pollacci omitted phosphorus from the

nutrient solution containing the pyrrole salt, solution *A* plus 0.250 g. of the pyrrole salt was also used. The cultures were made in 500-cc. wide-mouth bottles wrapped in black paper and provided with paraffined paper tops. Two 5-day-old seedlings were placed in each culture.

After 2 weeks' growth the corn plants in the solutions containing the pyrrole salt were decidedly stunted in size. The leaves of these plants were a deep bluish green and the culms dark red, while the roots were discolored and the development of lateral roots was greatly restricted. The plants in solution *A*, lacking iron, were growing satisfactorily, but the leaves were a lighter green than those of the plants in solution *C*, containing iron. The nutrient solutions were renewed, but the concentration of the pyrrole salt was reduced to one half the amount originally employed.

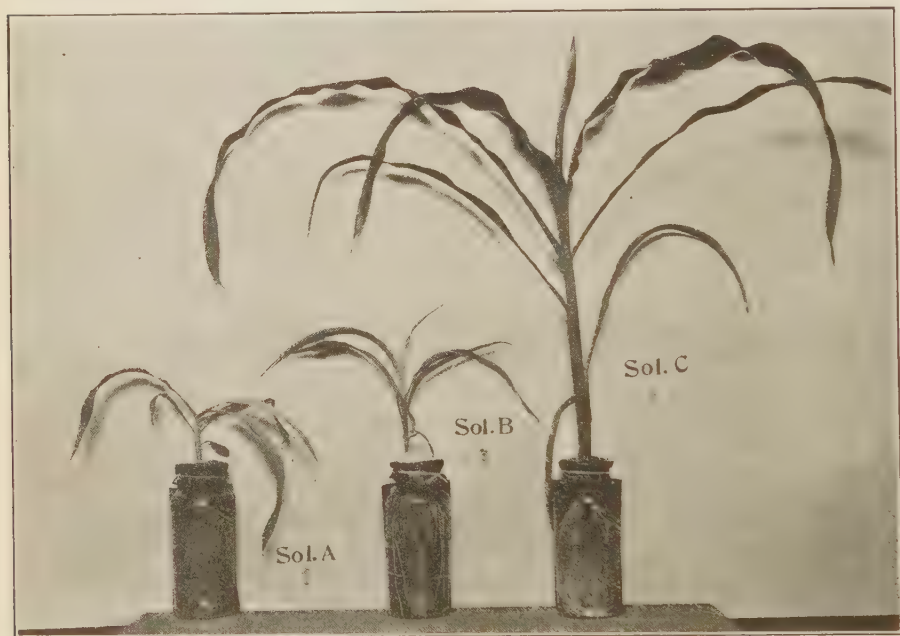
In the course of another week the roots of the plants in the solutions containing the pyrrole salt were injured to such an extent that the leaves rolled and wilted from lack of a proper water supply. This condition was overcome partially by placing the developing brace roots in the solutions. By the end of 7 weeks, only 3 of the plants receiving the pyrrole salt were still alive. These plants were very small, the new leaves were chlorotic, and the root systems were discolored. The development of lateral roots was particularly restricted. No difference was observed between the plants receiving phosphorus with the pyrrole salt and those without this element.

The plants in the solution minus iron were stunted in growth and the leaves were chlorotic, but the root systems were white and well branched. The plants supplied iron were 3 to 4 times the size of those lacking this element or supplied the pyrrole salt. The leaves of these plants were dark green and the root systems were in healthy condition. The appearance of representative plants at this time is shown in text figure 1.

The results of this experiment demonstrated that the pyrrole salt made in our laboratory was toxic to corn plants at concentrations of 0.125 and 0.250 g. per liter, both in solutions containing phosphorus and in those without this element. A reduction of the concentration of the pyrrole salt by one half did not allow the plants to recover from their injured condition. The leaves of the plants first acquired a bluish-green color accompanied by increased anthocyanin content in the culms and sheaths, but later the new leaves became chlorotic. The roots of the plants were most severely injured, in many cases to the extent of not being able to supply sufficient water to the leaves.

Possible explanations for the injurious effects obtained are: (1) that the pyrrole salt employed differed from that used by Oddo and Pollacci; (2) that toxic impurities were present; (3) that the concentration of the pyrrole salt was too high; (4) that the action of microorganisms upon this complex organic salt might produce injurious decomposition products; (5) that the plants used were too young. The method of preparation of the pyrrole

salt, its decomposition temperature, and the analysis cited indicate that the material used was the magnesium salt of pyrrole carbonic acid. The compound was purified in all cases in the manner described by Oddo and Pollacci, and in several of the later experiments it was purified twice. The other factors mentioned as being probable causes of the injurious effects were investigated.



TEXT FIG. 1. Reid's Yellow Dent corn grown 7 weeks in solution *A*, lacking iron, solution *B* containing 0.125 g. pyrrole salt per liter, and solution *C* containing 0.030 g. ferric chlorid per liter.

The concentration at which the pyrrole salt proved toxic in the experiment described above is that which Pollacci and Oddo used in their experiments. Nevertheless it was thought advisable to determine the effect of reducing the concentration.

EFFECT OF REDUCING THE CONCENTRATION OF THE PYRROLE SALT

Green and yellow seedlings of a strain of corn (5463-13 $\otimes$) kindly supplied by Dr. E. W. Lindstrom were grown with the pyrrole salt at several concentrations both with and without phosphorus. In solution *B* the pyrrole salt was used at rates of 0.025, 0.050, and 0.125 g. per liter, and in solution *A* at rates of 0.025, 0.050, 0.125, and 0.230 g. per liter. The check solutions *A* and *C* were also maintained, the latter containing 0.03 g. ferric chlorid per liter. Two 6-day-old seedlings, one green and one yellow, were placed in each culture.

In 10 days the green seedlings in all the solutions containing the pyrrole salt were slightly chlorotic, while those in solution *A* were distinctly chlorotic. The yellow seedlings showed no tendency to develop chlorophyll either in the solutions containing the pyrrole salt or in the solution with iron. When the solutions were renewed in 2 weeks, the root systems of all the plants in the solutions containing more than 0.025 g. of pyrrole salt per liter were discolored and the lateral roots were stubby and swollen at the tips.

The yellow seedlings in all the solutions grew very little after the first 2 weeks. The leaves of these plants became water-soaked, and within another 2 weeks all had died without becoming green.

When the experiment was concluded, in 4 weeks, the green plants in solutions containing 0.125 and 0.23 g. pyrrole salt per liter were stunted in growth, the leaves were chlorotic, and the roots were severely injured. With the lesser concentrations of this salt, 0.025 and 0.050 g. per liter, the growth of the plants was stunted and the leaves were chlorotic, but the roots were much less injured than in the solutions with the higher concentrations. In general, the plants in the solution lacking phosphorus, solution *B*, were in a slightly better condition than those in solution *A*, containing phosphorus and the pyrrole salt. The plants grown without iron were very chlorotic while those supplied with iron were large and normal in all respects.

The results of this experiment show that a reduction of the concentration of the pyrrole salt to 0.025 g. per liter decreased the injury to the corn plants but did not prevent the green plants from becoming chlorotic nor did it induce chlorophyll-development in the yellow plants. The growth of the green plants supplied with iron was greatly superior to the growth of those supplied with the pyrrole salt. The presence of phosphorus increased the injury caused by the pyrrole salt.

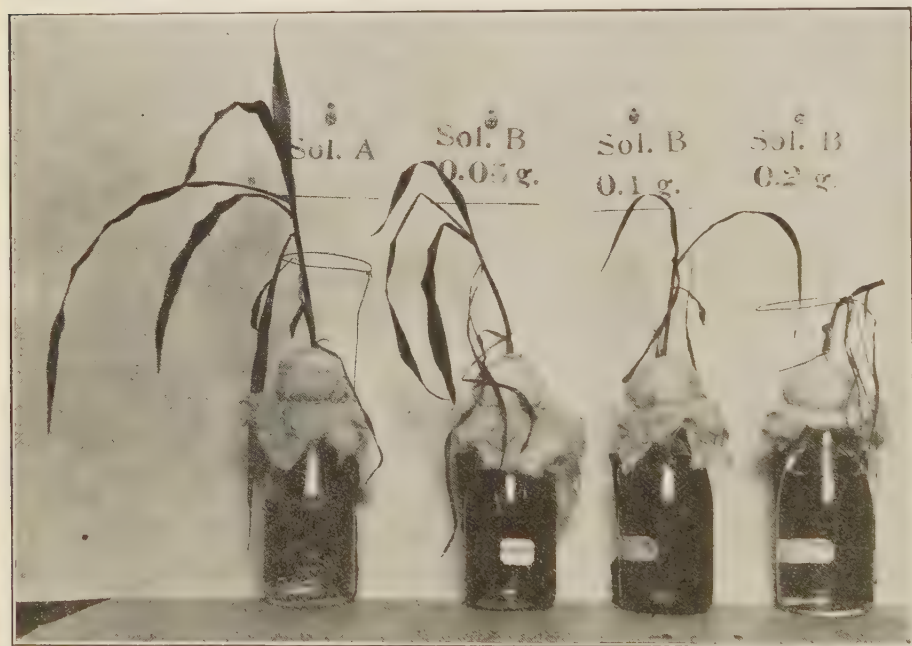
In the above-described experiment it was observed that the nutrient solutions containing the pyrrole salt became turbid. This suggested the presence of bacteria. A few drops of the turbid nutrient solution were transferred to a freshly prepared culture of solution *A* containing 0.125 g. pyrrole salt per liter. In 36 hours at 28 C. the solution became turbid, and microscopic examination showed the presence of rod-shaped bacteria. The possibility of bacterial activity producing toxic decomposition substances from the pyrrole salt was at once apparent. To eliminate the activity of bacteria, experiments were carried out under sterile conditions.

EXPERIMENTS WITH CORN PLANTS SUPPLIED THE PYRROLE SALT UNDER STERILE CONDITIONS

The methods of Wilson (7) for growing large plants in sterile media were used. Some of the nutrient solutions were sterilized by autoclaving at 18 lbs. pressure for 15 minutes, while others were sterilized in an Arnold

sterilizer for 30 minutes on 3 successive days to avoid the possibility of decomposing the complex pyrrole salt by the high temperatures of the autoclave. Longfellow flint corn was used in all the trials. Solution *B* containing 0.05 to 0.23 g. of pyrrole salt per liter, and solution *A*, lacking iron, were used in all these tests.

The results of every trial with the pyrrole salt in sterile solutions showed that it was as toxic under these conditions as under non-aseptic conditions. Text figure 2 illustrates the appearance of a typical series of corn plants grown under sterile conditions for 30 days.



TEXT FIG. 2. Longfellow flint corn grown 30 days with the roots under sterile conditions in solution *A*, lacking iron, and in solution *B* containing 0.05, 0.10, and 0.20 g. pyrrole salt per liter respectively.

Although Oddo and Pollacci used corn plants in their experiments with the pyrrole salt, it was thought that more favorable results might be secured with other plants since all the work with corn had given negative results. Experiments were therefore performed with cowpea, soybean, and *Spirodela*. At the same time that cowpea plants were grown in solutions containing the pyrrole salt, some of the plants were treated with a solution of this salt applied to the leaves as a paint.

THE INFLUENCE OF THE PYRROLE SALT UPON COWPEA AND SOYBEAN PLANTS

New Era cowpea seedlings and Wilson soybean seedlings were grown in solution *B* containing 0.10 g. pyrrole salt per liter, in solution *A*, lacking iron, and in solution *C* containing 0.01 g. per liter of ferrous sulfate. Two cultures of solution *B* minus the pyrrole salt were set up so that this salt could be applied to the leaves.

When the experiment was concluded, after 21 days' growth, the toxicity of the pyrrole salt at a concentration of 0.10 g. per liter to both cowpea and soybean plants was evident. The most severe injury was sustained by the roots of the plants, while the leaves became chlorotic or died early. When the pyrrole salt in aqueous solution was applied to the leaves of cowpea plants the majority of the developing leaves were killed, a few became chlorotic, and one became a light mottled green. This method of supplying the pyrrole salt did not injure the root systems.

THE USE OF RELATIVELY DILUTE CONCENTRATIONS OF THE PYRROLE SALT WITH COWPEA PLANTS

It was thought that beneficial effects of the pyrrole salt might be obtained in concentrations low enough to escape its toxic action. Six-day-old cowpea seedlings were therefore grown in solution *B* containing the pyrrole salt at rates of 0.001, 0.005, 0.010 and 0.020 g. per liter. The solutions were renewed every 3 days.

Within 10 days the leaves of the plants in all the solutions containing the pyrrole salt were yellow-green. A few plants formed several compound leaves, but they were small, malformed, and chlorotic with a slight tendency toward greenness about the main veins. The lateral roots of the plants receiving 0.010 and 0.020 g. pyrrole salt were very short and swollen at the tips. All the plants receiving the pyrrole salt died within 4 weeks. Reducing the concentration of the pyrrole salt reduced its toxicity but did not permit the development of chlorophyll in the absence of iron.

GROWTH OF SPIRODELA POLYRHIZA WITH THE PYRROLE SALT

Plants of *Spirodela polyrhiza*, a floating aquatic, were used in one experiment to observe the influence of the pyrrole salt on mature plants since the previous work had been with seedlings. A concentration of 0.10 g. pyrrole salt per liter was used in solution *A* diluted 10 times and checked with solution *A* minus iron and solution *A* containing ferric chlorid at the rate of 0.010 g. per liter.

Injury to these plants was noticeable after 4 days, many of the leaves becoming yellow to brown in color. The roots developed dark-colored swellings near the tips, lacked the normal chlorophyll content, and soon became flaccid. The experiment was concluded after 25 days. Little

growth had been made by the plants in the pyrrole solution, while many had died and those still remaining were yellow and very small. The roots of the majority of the plants were decaying. The plants in the solution lacking iron had made fair growth but the leaves were chlorotic, while those supplied with iron made excellent growth and were normal in every respect.

EXPERIMENTS WITH ODDO'S PYRROLE SALT

Early in the present investigations, when it was found that the pyrrole salt used was injurious to corn plants, the writer communicated with Professor Oddo to see if he had experienced a similar difficulty or could explain the reason for the toxic action. He was of the opinion that toxic impurities were present in our compound and emphasized the necessity of protecting the pyrrole salt from light and renewing the nutrient solutions often. He also kindly supplied a sample of his compound.

Using this salt, an experiment was conducted with cowpea plants that had been grown 14 days in a nutrient solution lacking iron. The first compound leaves of these plants were about half developed and were only slightly lighter green than the primary simple leaves. The Oddo and Missouri pyrrole salts were used at concentrations of 0.20 g. per liter in solution *A* and checked with solution *A* minus iron, and with the same solution containing 0.005 g. iron as ferric chlorid. The solutions were renewed every 4 days.

In 4 days it was observed that the new leaves of the plants in the pyrrole salt solutions were slightly lighter green than those of the plants receiving iron. On the following day the primary leaves began to become yellow. By the seventh day the plants receiving pyrrole salt had lost their primary leaves, and the compound leaves remained small, were yellowish green, dying at the tips and around the margins, and several were completely wilted. These signs of injury were slightly more advanced in the plants growing in the solution containing the Missouri pyrrole salt.

The plants in the pyrrole salt solutions were so severely injured by the eighth day that the experiment was discontinued. In the solution containing Oddo's pyrrole salt 2 plants were completely defoliated, and one retained a compound leaf of light green color injured at the tips and around the margins of the leaflets. The roots of these plants were slightly discolored and flaccid. The condition of the plants in the solution containing the Missouri pyrrole salt was practically the same as described for those in the solution containing Oddo's compound. The only compound leaf remaining was very light green in color. The plants supplied with iron were in a normal growing condition, each having 2 or 3 well developed dark green compound leaves and turgid, white root systems. The plants lacking an iron supply had light green and white compound leaves but normal roots.

A very heavy growth of bacteria was observed in the solutions con-

taining the pyrrole salts, causing the solutions to be turbid and zoöglæa to form on the surface. Bacterial decomposition of the pyrrole salts may have markedly increased the injury to the plants. The solutions without the pyrrole salts were clear.

In a second experiment the Oddo and Missouri pyrrole salts were used at a concentration of 0.10 g. per liter in solution *A* with 6-day-old Long-fellow flint corn seedlings. The solution containing Oddo's compound was renewed on the fifth and ninth days. The supply of the Missouri compound was exhausted, so this solution was not changed.

During the first 5 days no differences were noticed between the plants supplied the pyrrole salts and those without it or supplied with iron. By the ninth day the tops of the plants supplied with the pyrrole salts appeared slightly smaller and a trifle more green than the check plants. The roots, however, were decidedly smaller than those of the check plants and were slightly discolored and flaccid.

A growth of molds developed about the corn grains, so the endosperms were removed. By the thirteenth day the leaves of the plants in the pyrrole solutions were badly wilted. An examination of the root systems showed those of the plants in the pyrrole solutions to be flaccid and about one half to two thirds the length of the check plants. Some of the lateral roots had swollen tips. The solutions containing the pyrrole salts were turbid, this condition indicating bacterial growth.

DISCUSSION

The results of the experiments performed by the writer fail to confirm the ability of a magnesium salt of pyrrole carbonic acid to replace iron in the development of chlorophyll by plants. They show, on the contrary, that the compound is quite toxic at concentrations considerably less than those reported by Oddo and Pollacci at which plants developed normally.

Why the results reported by the writer and those reported by Oddo and Pollacci should be so divergent can not be explained. All possibilities save those of different environments have apparently been eliminated. The experiments with the salt synthesized at the University of Missouri have taken into account the purification of the pyrrole salt, suitable nutrient solutions, the concentration of the pyrrole salt, the exclusion of light from the solutions, the renewal of the solutions, the age of the plant material, the action of microorganisms on the organic salt, and variation in kinds of plants. There still remained the possibility that the salt synthesized at the University of Missouri, although agreeing in empirical formula with the theoretical, might be an isomer of that used by Oddo and Pollacci. The limited experiments performed with the salt secured from Oddo show, however, that it acts upon plants under our conditions like the material prepared here.

SUMMARY

1. Corn, cowpea, soybean, and Spirodela plants were grown in nutrient solutions containing a magnesium salt of pyrrole carbonic acid substituted for iron. In no case did this compound prevent chlorosis of the leaves of the plants. This result is contrary to those reported by Oddo and Pollacci.
2. In water cultures the pyrrole salt at concentrations of 0.001 to 0.250 g. per liter was toxic to the plants used.
3. The pyrrole salt was toxic to cowpea plants when applied to the leaves as a paint.
4. The decomposition point and analysis of the pyrrole salt used in this investigation indicated that it was the same salt as that used by Oddo and Pollacci.
5. Microorganisms develop luxuriantly in a nutrient solution containing the magnesium salt of pyrrole carbonic acid.
6. Under sterile conditions the pyrrole salt proved toxic to corn plants.
7. A sample of the pyrrole salt secured from Oddo was toxic to cowpea plants at a concentration of 0.20 g. per liter and to corn plants at a concentration of 0.10 g. per liter.

The writer wishes to express his sincere thanks to Dr. W. J. Robbins for many helpful suggestions and for his close interest in this investigation.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MISSOURI

LITERATURE

1. Ciamician, G., and Galizzi, A. The behavior of some organic substances in plants XIV. *Gaz. Chim. Ital.* 52: 1-20. 1922. Also in *Chem. Abst.* 16: 1972. 1922.
2. Oddo, B., and Moschini, A. Sintesi nel gruppo del pirrolo. Nota VII: Derivati dell'acido α -pirrolcarbonico ed acido β -pirrolcarbonico. *Gaz. Chim. Ital.* 42: 244-256. 1912.
3. —, and Pollacci, G. Influenza del nucleo pirrolico nella formazione della clorofilla. *Gaz. Chim. Ital.* 50: 54-70. 1920.
4. Pollacci, G., and Oddo, B. Influenza del nucleo pirrolico sulla formazione della clorofilla. Nota preliminare. *R. Acad. Lincei* 24: 37-39. 1915.
5. Willstätter, R. Zur Kenntniss der Zusammensetzung des Chlorophylls. *Ann. Chem.* 350: 48-82. 1906.
6. —, and Asahina, Y. Ueber die Reduktion des Chlorophylls. *Liebig's Ann. Chem.* 385: 188-226. 1911.
7. Wilson, J. K. Device for growing large plants in sterile media. *Phytopath.* 10: 425-439. 1920.

POTASSIUM FERROCYANIDE AND FERRIC FERROCYANIDE AS SOURCES OF IRON FOR PLANTS

C. G. DEUBER

University of Missouri

Received for publication July 28, 1925

In search of a compound that would furnish iron satisfactorily to plants grown in weakly acid or slightly alkaline nutrient solutions a number of experiments were performed with potassium ferrocyanide and ferric ferrocyanide.¹ Potassium ferrocyanide is unique as an iron source because it contains iron in the anion. Ferric ferrocyanide, or Prussian blue, contains iron in both the cation and anion and is relatively insoluble in water. These complex iron salts were found to be fair sources of iron for green plants under rather limited conditions. With potassium ferrocyanide the chief condition was to secure a low enough concentration of the salt to escape its toxic effects, whereas with ferric ferrocyanide the reaction of the nutrient solution was the chief factor determining the availability of the iron.

Knop (1) in 1869 found that chlorotic corn plants became green when supplied with potassium ferrocyanide at the rate of 0.1 per mille (13.22 p.p.m. of iron) but that the growth of the plants was slowed and finally stopped. Wagner (8) in the following year confirmed the results of Knop and observed a slight deposit of ferric ferrocyanide on the roots. Susuki (5) observed a poisonous action on barley seedlings of potassium ferrocyanide at a concentration of 0.01 per mille (1.322 p.p.m. of iron) in water cultures. In soil cultures (6) it stimulated growth. He ascribed the poisonous action to hydrocyanic acid, which was formed within the plant by the splitting of the potassium ferrocyanide, but was unable to decide whether the stimulating action in the soil was due to the compound itself or to its decomposition products. Loew and Kozai (2) observed a stimulating effect of 0.01 per cent potassium ferrocyanide on *Bacillus prodigiosus* but no improvement in the growth of other bacteria. No reference to the use of ferric ferrocyanide as a source of iron for plants was found in the literature.

EXPERIMENTAL

Spirodela polyrhiza (L.) Schleid. plants, floating aquatics, were grown in Knop's nutrient solution diluted 10 times in accordance with the findings of Saeger (4). Iron was added to the solution in the forms of ferric citrate,

¹ The author wishes to acknowledge his thanks to Dr. W. J. Robbins for helpful criticism and advice in the course of this investigation.

ferrous sulfate, ferric chloride and potassium ferrocyanide at the concentrations indicated in table 1. To one solution no iron was added.

The three commonly used iron salts: ferric citrate, ferrous sulfate, and ferric chloride produced a very satisfactory growth of these plants, the largest growth being secured with ferrous sulfate as is shown in table 1. The leaves of the plants in the solution containing the lowest concentration of iron as potassium ferrocyanide (0.016 p.p.m. iron) were small and chlorotic. The plants in the solutions containing 0.033 and 0.066 p.p.m. iron as potassium ferrocyanide made fair growth but the leaves were a trifle lighter green than those of plants in the solutions containing the commonly used iron salts.

TABLE I

*Growth data of Spirodela plants grown 23 days in Knop's solution containing iron in the salts as the concentrations indicated.**

IRON SALT	CONCENTRATION OF IRON	AVERAGE NUMBER MATURE LEAVES PER CULTURE†	AVERAGE GREEN WEIGHT PLANTS PER CULTURE	REACTION OF SOLUTIONS	
				Initial	Maximum final
	<i>p.p.m.</i>		<i>mgm.</i>	<i>pH</i>	<i>pH</i>
Check.....	0.000	18.25	37.00	6.6	7.4
Fe citrate.....	2.280	152.25	513.75	6.4	7.3
FeSO ₄	3.676	160.75	563.75	6.5	7.1
FeCl ₃	2.065	137.25	527.00	6.2	7.0
K ₄ Fe(CN) ₆	0.016	62.00	140.00	6.7	7.4
	0.033	102.00	275.00	6.7	7.6
	0.066	93.50	308.75	6.7	7.6
	0.132	78.50	267.25	6.7	7.7
	0.264	53.50	171.25	6.6	7.5

* The nutrient solutions were renewed 5 times at intervals of 4 days.

† Average of 4 cultures.

With the highest concentrations of iron as potassium ferrocyanide (0.132 and 0.264 p.p.m. iron) the leaves of the plants were normal green but the growth of the plants with the latter concentration was greatly reduced. The plants in the solution without iron made very poor growth and the leaves were chlorotic.

With soybean plants concentrations of 0.264 p.p.m. and higher of iron in the form of potassium ferrocyanide greatly depressed growth. Concentrations of 0.033 and 0.066 p.p.m. iron in this iron salt produced fair growth of soybean plants, but chlorophyll development was not adequately provided for, the leaves being light green.

Potassium ferrocyanide was used in solutions having reactions from pH 3.1 to 8.3. *Spirodela* plants grew best at pH 6.2 and 6.8. This iron salt was not so satisfactory at neutral and slightly alkaline reactions as ferric citrate.

Merck's iron ferrocyanide used at concentrations of 0.022 to 9.0 p.p.m. iron per liter in Knop's solution resulted in very poor growth and chlorosis of the

foliage of soybean and *Spirodela* plants. The explanation of these results was found when this iron salt was used in a series of buffered solutions having reactions of pH 4.0 to 8.3. Knop's solution was modified by omitting KNO_3 , substituting H_3PO_4 at the rate of 0.8055 gm. per liter for KH_2PO_4 , and adding potassium acid phthalate at the rate of 1.0207 gm. per liter. These changes were based on the buffered nutrient solution developed by Tarr and Noble (7). At pH 5.0 soybean plants made a greater growth when the solution contained ferric ferrocyanide than when it contained ferric citrate, the iron contents of both solutions being the same (5 p.p.m. iron). At pH 5.5 the foliage of the plants in the solution containing ferric ferrocyanide was light green and at all higher reactions it was distinctly chlorotic. The plants in the solutions con-

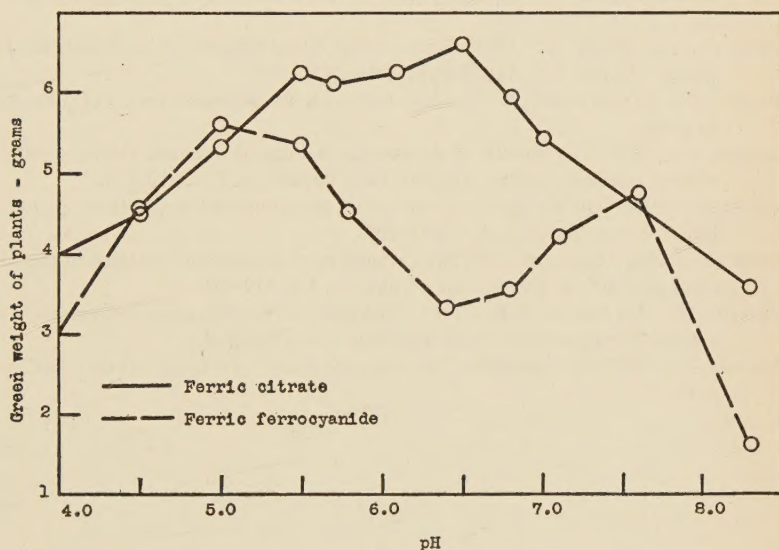


FIG. 1. GREEN WEIGHT OF SOYBEAN PLANTS FOR EACH CULTURE IN NUTRIENT SOLUTIONS CONTAINING 5 P.P.M. IRON AS FERRIC CITRATE AND FERRIC FERROCYANIDE

taining ferric citrate made very satisfactory growth from pH 5.5 to 6.5, but from pH 6.8 to 8.3 the leaves of the plants were chlorotic.

The reaction of Knop's solution as used for soybeans in the earlier experiments was pH 5.8 and when diluted 10 times for *Spirodela* plants, pH 6.6. Both of these reactions were in the region in which the iron of ferric ferrocyanide in the above experiment was insufficiently available for the normal growth of soybean plants.

The growth curve (fig. 1) of the soybean plants grown in the buffered nutrient solutions containing ferric ferrocyanide shows two maxima, one occurring at pH 5.0 and a second at pH 7.6 with a marked depression in growth around pH 6.4. It is of interest to note that Robbins and Scott (3) found the isoelectric point of soybean root tips to be in the vicinity of pH 6.4.

SUMMARY

1. With 0.033 and 0.066 p.p.m. iron in the form of potassium ferrocyanide, soybean and *Spirodela polyrhiza* plants made fair growth. Higher concentrations of iron in this salt produced a slow stoppage of growth.

2. Merck's ferric ferrocyanide was a satisfactory source of iron for soybean plants when the solution had a reaction of pH 5.0 but at less acid reactions growth of the plants and chlorophyll development was restricted.

REFERENCES

- (1) KNOP, W. 1869 Versuche über die Wirkung der Eisensalze auf das Ergrünen der Chlorophyllkörner. Ber. Ver. Königl. Sächs. Ges. Wiss. Math. Phys. Cl. Leipzig, v. 21, p. 1-27.
- (2) LOEW, O., AND KOZAI, Y. 1902 Ueber Ernährungsverhältnisse beim *Bacillus prodigiosus*. In Bul. Col. Agr. Tokyo, v. 5, p. 137-141.
- (3) ROBBINS, W. J., AND SCOTT, I. Further studies on the isoelectric points of plant tissue. In press.
- (4) SAEGER, A. 1925 The growth of duckweeds in mineral nutrient solutions with and without organic extracts. In Jour. Gen. Physiol., v. 7, p. 517-526.
- (5) SUSUKI, S. 1902 On the poisonous action of potassium ferrocyanide on plants. In Bul. Col. Agr. Tokyo, v. 5, p. 203-205.
- (6) SUSUKI, S. 1903 Can potassium ferrocyanide exert any stimulant action in the soil on plant growth? In Bul. Col. Agr. Tokyo, v. 5, p. 517-518.
- (7) TARR, L. W., AND NOBLE, S. C. 1922 The effect of hydrogen-ion concentration upon the growth of seedlings. Del. Agr. Exp. Sta. Tech. Bul. 1.
- (8) WAGNER, P. 1870 Wasserculture-Versuche mit Mais. In Landw. Vers. Stat., v. 13, p. 69-75.

